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Human α-Thalassemia Syndromes: Detection of Molecular Defects

Antonios C. Kattamis, Clara Camaschella, Piera Sivera, Saul Surrey, and Paolo Fortina

Division of Hematology, Department of Pediatrics, Children's Hospital of Philadelphia, University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania (A.C.K., S.S., P.F.); Dipartimento di Scienze Biomediche ed Oncologia Umana, Sezione Clinica, Università degli Studi, CNR-CIOS, Torino, Italy (C.C., P.S.)

INTRODUCTION

The human α-like globin gene cluster is located on the distal portion (p13.3–pter) of the short arm of chromosome 16 and includes one embryonic gene (ζ), three pseudogenes (ψζ, αζ1, ψα2), two adult genes (α_2 and α_1), and one gene (θ_1) of unknown function, arranged in the order 5′- ζ-ψζ-ψα_2-ψα_1-α_2-α_1-θ_1-3′. The two highly homologous α_2 and α_1 genes are embedded in two 4-kb DNA units which arose by a duplication event, and are each divided into three homologous subsegments (X, Y, and Z), separated by three nonhomologous segments (I, II, and III) [1,2].

α-Thalassemia is caused by deficient synthesis of α-globin chains. The molecular lesions can be categorized into α^+ or α^0 defects, depending on whether they partially or completely abolish α-globin chain production, respectively. A wide variety of point mutations and deletions has been described which decrease α-globin gene expression in humans [1,2]. Clinical phenotype of the carriers varies according to the number of affected genes. Carriers of three α-globin genes ($-\alpha/\alpha\alpha$) present with no detectable red blood cell abnormalities or globin-chain imbalance, while carriers of two functioning α-genes ($--/\alpha\alpha$ or $-\alpha/-\alpha$) have mild microcytic, hypochromic anemia with normal hemoglobin A_2 levels. Carriers of only one functioning α-gene ($-\alpha/--$) present with hemoglobin H (β_4 tetramers) disease, which is characterized by severe anemia with markedly unbalanced globin-chain synthesis ratios (mean, 0.43) [1]. Inheritance of no functional α-globlin genes ($--/--$) is usually incompatible with life and leads to Hb Bart's (γ_4 tetramers) hydrops fetalis.

Although α-thalassemia is found throughout the world, its distribution varies greatly among different populations. The prevalence of deletion of one α-globin gene is high in most populations of the malaria belt, reaching over 80% in some areas of India, Nepal, and the Southwest Pacific, suggesting a protective effect against this parasitic infection. Deletion of both α-globin genes on the same chromosome and nondeletional forms of α-thalassemia are less common and are found, in particular, in Southeast Asians and Mediterraneans [3]. The spectrum of molecular defects is somewhat ethnic-specific; although the common mutations in different endemic areas have been characterized, the repository of sporadic mutations continues to expand.

Characterization of the molecular defects of deletional forms of α-thalassemia has for a long time been achieved using restriction-enzyme analysis of genomic DNA followed by Southern blotting and hybridization with radiolabeled α-globin gene probes. The advent of the polymerase chain reaction (PCR) has revolutionized molecular biology and simplified detection of the most common variants of α-thalassemia.

Previous reviews have focused on epidemiology, genetics, and clinical characteristics of thalassemia. Since the last published review, newly-characterized mutations have been presented [2]. In this review, we catalogue the various molecular defects causing α-thalassemia according to their geographical-ethnic distribution, and emphasize methods employed for their detection. We hope to provide a technical guide for the diagnosis of the various α-thalassemia syndromes which will facilitate population screening, genetic counseling, and research on the thalassemias. Nomenclature for probes and primers is as described in the original references.

TECHNIQUES

Gene Mapping

The conventional approach to characterizing α-thalassemia deletions involves gene mapping and Southern blot hybridization using radioactively-labeled probes. A wide variety of probes spanning the entire α-like globin gene cluster has been described, with the α- and ζ-globin gene probes being the most commonly used (Table I).

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Address reprint requests to Paolo Fortina, M.D., Abramson Pediatric Research Center, Room 310C, Children's Hospital of Philadelphia, 34th Street and Civic Center Boulevard, Philadelphia, PA 19104.

PCR-Based Strategies

Different PCR-based approaches have recently been applied for detection of the most common deletional and non-deletional forms of α-thalassemia [4–24]. Compared to conventional restriction-enzyme analysis, PCR technology is simpler, more reliable, rapid, and user-friendly (Table II). Many strategies for the detection of common non deletional forms involve selective amplification of the α_2- or α_1-globin genes. PCR products can then either be blotted to nylon membranes and hybridized to radiolabeled, allele-specific oligonucleotide (ASO) probes (Table III), or they can be digested with a restriction endonuclease, in instances where the mutation creates or abolishes a cleavage site (Table IV). When these approaches fail, direct characterization of the mutation can be achieved by DNA sequence analysis employing either manual or automated sequencing strategies.

Selective amplification of mutant or normal alleles by specific oligonucleotide primers has been applied for the detection of both deletional and nondeletional forms [6–9]. In the case of deletion, oligonucleotide primers representing unique sequences from the two regions flanking the deletion are used to selectively amplify the region spanning the deletion breakpoint. Amplification of the region using these primers in normal controls does not normally occur, since the two priming sites are separated by too great a distance. In patients with the deletion, this distance is shorter, and primers can be chosen so that a unique PCR product is generated which is characteristic for each deletional form of α-thalassemia. A second primer pair can be used which maps within the deleted region so that only the normal allele is amplified in heterozygotes as an internal control.

Single-strand conformation analysis (SSCA), in which detection is based on differences in the electrophoretic pattern of single-stranded DNA from mutant and normal alleles, and multiplex PCR, which compares different regions of the cluster amplified in the same reaction, have also been applied as screening methods for the identification of point mutations and gene deletions [10,22].

MOLECULAR DEFECTS

The spectrum of α-thalassemia alleles includes deletional and nondeletional forms. One group of deletions removes the locus control region (LCR) and silences the locus, which remains structurally intact [25–29]. The LCR is located between 33–55 kb 5′ of the α-like globin gene cluster, and encompasses sequences which are critical for the regulated expression of the cluster. All other deletions remove either one (α^+/α-thalassemia-2) or both α-globin genes (α^0/α-thalassemia-1). α-Thalassemia-1 deletions are of limited geographic distribution and, except for $--^{SEA}$, $-(\alpha)^{20.5}$, and $--^{MED}$, are sporadic and detected in isolated families. PCR-based methods have been described for detection of the most common deletions ($-\alpha^{3.7}$, $-\alpha^{4.2}$, $--^{SEA}$, $--^{MED}$, $-(\alpha)^{20.5}$), and are based on selective amplification of normal or mutant alleles [5–9].

Nondeletional forms of α-thalassemia involve point mutations or deletion/insertion of a few nucleotides, resulting in decreased expression of α-globin genes. In contrast to β-thalassemia, these mutations constitute the minority of molecular defects leading to α-thalassemia. Almost all defects described affect expression of the α_2 gene, which probably represents a selection bias, as the α_2 gene encodes 2–3 times more mRNA than the α_1 gene [30]. Many mutations lead to production of α-globin chain variants, of which we have included those that are associated with a significant clinical phenotype.

Detection of nondeletional forms of α-thalassemia is facilitated by an initial step of selective amplification of the α_2 and α_1 genes using the C1/C3 and C1/C9c oligonucleotide primer pairs, respectively (Table II). Selectivity of amplification can be confirmed by digestion of the PCR product with *Apa*I, which gives distinctly different size bands for each α gene [4]. The spectrum of defects is summarized in Table IV.

The different α-thalassemia alleles are presented and grouped below according to ethnic/geographic distribution, based on published epidemiological studies. Population migration, racial mixture, and unavailability of accurate estimates for the frequency of each allele need to be considered when using this catalogue. Some deletions can be detected by Southern blot analysis, provided that a probe is available which flanks the deletion and can be used to detect a unique fragment which spans the deletion breakpoint. Data summarized in Table I show aberrant band size, restriction enzymes, and probes used for detection of different deletional forms of α thalassemia. In cases where no aberrant bands are obtained with the use of available probes, the presence of deletions following restriction mapping is suggested by decreased signal intensity of normal bands and by inheritance of informative neutral sequence polymorphisms. A diagrammatic summary of deletional forms of α-thalassemia encompassing the α-like globin gene cluster is presented in Figure 1.

WORLDWIDE

The α-thalassemia-2 (α^+) deletions are the most common forms of α-thalassemia, with two major forms predominating ($-\alpha^{3.7}$ and $-\alpha^{4.2}$) which are found throughout the world.

$-\alpha^{3.7}$

This 3.7-kb deletion, which is also known as rightward α-thalassemia-2, is the result of homologous recombina-

TABLE I. Detection of Deletional Forms of α-Thalassemia by Southern Blot Analysis*

Allele	*Bam*HI [Probe]: kb	*Bgl*II [Probe]: kb	*Eco*RI [Probe]: kb	*Hind*III [Probe]: kb	*Hpa*I [Probe]: kb	*Xba*I [Probe]: kb	Reference no.
Asians							
$-\alpha^{4.2}$	[α]: 10.0		[α]: ~19		[α]: 4.2		31
$-(\alpha)^{2.7}$	[α]: 11.5	[θ_1]: 4.7	[α]: 20.5	[α]: 5.4	[θ_1]: 12.5		35
$-(\alpha)^{3.5}$	[α]: 10.0	[α]: 4.1			[α]: 10.0		36
$--^{SEA}$	[ψζ]: 17–20		[ψζ]: 16.5	[ζ]: 13.5 [ψζ]: 17–20			33, 52
$--^{SA}$		[ζ]: 17.20	[3′-HVR]: 6.4		[3′-HVR]: 5.3		38, 39
$--^{THAI}$			[3′-HVR]: 8.0 [L_0]: 8.0	[3′-HVR]: 20.0 [L_0]: 20.0	[3′-HVR]: 9.6		40
$--^{FIL}$			[3′-HVR]: 4.0	[3′-HVR]: 17.5 [L_0]: 17.5	[3′-HVR]: 9.5 [L_0]: 9.5		40
Mediterraneans							
$\alpha^{3.7}$	[α]: 10.5	[α]: 16.0	[α]: 19.0				31
$-(\alpha)^{20.5}$	[α]: 3.9	[ζ]: 9.7 [α]: 9.7 [θ_1]: 9.7	[α]: 2.6 [θ_1]: 10.0				49, 52
$--^{MED}$		[ζ]: 13.9	[θ_1]: 16.5	[θ_1]: 6.8		[θ_1]: 3.9	50, 52
$--^{MED\ II}$		[θ_1]: 4.8	[θ_1]: 14.0	[θ_1]: 6.0		[θ_1]: 11.5	52
$(\alpha)\alpha^{5.3}$	[α]: 8.8	[α] 6.8 [ζ] 6.8		[α]: 10.7 [ζ]: 10.7	[α] 9.7 [ζ]: 9.7	[α]: 10.7 [ζ]: 10.7	53
$-(\alpha)^{5.2}$	[α]: 9.0	[α]: 14.0					54, 55
$--^{CAL}$	[L_1]: 6.0		[3′-HVR]: 13.0	[θ_1]: 7.0	[3′-HVR]: 11 [θ_1]: 11		56, 57
$--^{CI}$		[ζ]: 16.0				[ζ]: 19.0	58
$--^{CANT}$		[ζ]: 4.0	[ζ]: 17 [3′-HVR]: 17	[ζ]: 24 [3′-HVR]: 24	[ζ]: 16 [3′-HVR]: 16		59
$--^{SPAN}$	[3′-HVR]: 10.5	[ψζ]: 8.0 [ψα_1]: 8.0 [3′-HVR]: 6.5	[3′-HVR]: 23 [R_2]: 23	[3′-HVR]: 15 [ψα_1]: 13	[3′-HVR]: 7.0	[ψα_1]: 9.6	60
$--^{MA}$		[ζ]: 8.6 [θ_1]: 8.6			[ζ]: 7.5 [θ_1]: 17.5	[ζ]: 11 [θ_1]: 11	61
$--^{YEM}$			[ψζ]: 11.0	[ψζ]: 13.0	[ψζ]: 17.5	[ψζ]: 14.3	62
$(\alpha\alpha)^{IdF}$		[Iζ HVR]: smear				[Iζ-HVR]: smear	29
$(\alpha\alpha)^{MM}$			[ζ]: 12.3			[Iζ-HVR]: 18.5	28
Africans							
$--^{GEO}$	[ψα_1]: 8.5	[ψα_1]: 10.5	[ψζ]: 23 [ψα_1]: 23 [3′-HVR]: 23	[ψα_1]: 16.5		[ψα_1]: 12.5	72
Northern Europeans							
$-(\alpha)^{18+}$	[α]: 8.0	[ζ]: 23 [α]: 23	[ζ]: ~24 [α]: ~24			[ζ]: 25 [α]: 25	84
$--^{BRIT}$		[ζ]: 7.8	[ζ]: 6.7	[ζ]: ~24			78, 85
$--^{MC}$	[3′-HVR]: 9.8 [L_2]: 9.8		[3′-HVR]: 7.2	[3′-HVR]: >20	[3′-HVR]: ~15 [L_2]: ~15		86
$--^{RT}$						[L_0]: 14	87
$(\alpha\alpha)^{IJ}$	[L_4]: 5.2 [$RA_{1.0}$]: 5.2	[L_4]: >23 [$RA_{1.0}$]: >23	[L_4]: ~17 [$RA_{1.0}$]: ~17	[L_4]: 13 [$RA_{1.0}$]: 13	[L_4]: ~13 [$RA_{1.0}$]: ~13		27
$(\alpha\alpha)^{TI}$	[L_4]: 2.0						26
$(\alpha\alpha)^{RA}$	[ψζ]: 14 [RA_{330}]: 14	[ψζ]: 6.8 [RA_{330}]: 6.8	[ψζ]: >23 [RA_{330}]: >23	[ψζ]: 12.6 [RA_{330}]: 12.6			25

*Various α-globin gene alleles are listed on left, with restriction endonucleases at top, and probes [in brackets] used for detection of aberrant bands whose size is indicated in kb after blotting and hybridization to radiolabeled probes.

tion between misaligned homologous Z regions, and is most prevalent in Mediterraneans and Africans [31]. There are three different subtypes (I–III), depending on the exact location of the breakpoint. Two PCR-based detection strategies have been described. The first uses C10 and C9c primers to selectively amplify across the breakpoint of the $-\alpha^{3.7}$ allele or the normal α_1 gene, yielding two PCR products of 1.9 kb of 2.1 kb, respectively (Table II) [7]. The second strategy uses the A/C and A/B primer pairs to selectively amplify normal or

TABLE II. Oligonucleotide Primers Used for Selective Amplification of Normal (N) or Mutant (M) α-Globin Gene Alleles*

Allele	Primer pair	Product size	Primer sequence 5′–3′		Reference no.
α_1	C1 + C9c	1,093 bp	C1:	TGGAGGGTGGAGACGTCCTG	4, 7
	C10 + C9c	2.1 kb	C9c:	CCATGCCTGGCACGTTTGCTGAGG	
			C10:	GATGCACCCACTGGACTCCT	
α_2	C1 + C3	1,085 bp	C1:	TGGAGGGTGGAGACGTCCTG	4, 7, 9, 19
	C8 + C3	1,943 bp	C8:	GAGCCTGGCCAAACCATCAC	
	C10 + C3	1.9 kb	C3:	CCATTGTTGGCACATTCCGG	
	A + C	1.8 kb	C10:	GATGCACCCACTGGACTCCT	
			A:	CTTTCCCTACCCAGAGCCAGGTT	
			C:	CCATTGTTGGCACATTCCGGGACA	
$-\alpha^{3.7}$	C10 + C9c	1.9 kb	C9c:	CCATGCCTGGCACGTTTGCTGAGG	4, 9
	A + B	1.8 kb	C10:	GATGCACCCACTGGACTCCT	
			A:	CTTTCCCTACCCAGAGCCAGGTT	
			B:	CCCATGCTGGCACGTTTCTGAGG	
$-\alpha^{4.2}$	D + F (N)	581 bp	D:	CCTTCCTCTCACTTGGCCCTGAG	9
	G + F (N)	227 bp	F:	GGCACATTCCGGGACAGAGAGAA	
	D + E (M)	2,115 bp	G:	CCGGTTTACCCATGTGGTGCCTC	
	G + E (M)	1,761 bp	E:	CCCTGGGTGTCCAGGAGCAAGCC	
			G:	CCGGTTTACCCATGTGGTGCCTC	
$(\alpha)^{5.2}$	TP2 + DH3	438 bp	TP2:	TCCTTTCCCTACCCAGAGCCA	55
			DH3:	CCATGCTGGCACGTTTCTGAGG	
$(\alpha)\alpha^{5.3}$	F + R	580 bp	F:	CTGCCGTGTCTCAATCTCTG	53
			G:	CTCACCTTGAAGTTGACCGG	
$\alpha^{Agr}\alpha$	C8 + α^{29}	1,205 bp	C8:	GAGCCTGGCCAAACCATCAC	19
			α^{29}:	GAGGGAGCCTCACCTCACCG	
$\alpha^{CS}\alpha$	For-N + Rev	578 bp	For-N:	GCTGACCTCCAAATACCGTT	46
	For-M + Rev	578 bp	For-M:	GCTGACCTCCAAATACCGTC	
			Rev:	GTAAACACCTCCATTGTTGG	
α^{TSaudi}	C8 + SPA	1,897 bp	C8:	GAGCCTGGCCAAACCATCAC	19
			SPA:	GCTGCCGCCCACTCACACC	
$-(\alpha)^{-20.5}$	2 + 3 (N)	1.07 kb	2 (C1):	TGGAGGGTGGAGACGTCCTG	6
	1 + 3 (M)	1.18 kb	1:	GGCAAGCTGGTGGTGTTACACA	
			3:	CCATGCTGGCACGTTTCTGAGG	
$--^{MED}$	5 + 6 (N)	1.0 kb	5:	TACAGCAGAGTGAGTGCTGCAT	6
	4 + 6 (M)	0.65 kb	6:	GGAGAAGTAGGTCTTCGTGGC	
			4:	ACAGTCACTCCTGAGGCCAGTC	
$--^{SEA}$	7 + 8 (N)	0.98 kb	7:	CTCTGTGTTCTCAGTATTGGAG	5, 6
	A + B (N)	314 bp	8:	TGAAGAGCCTGCAGGACCAGTCA	
	7 + 9 (M)	0.66 kb	A:	GCGATCTGGGCTCTGTGTTCT	
	A + C (M)	195 bp	B:	GTTCCCTGAGCCCCGACACG	
			9:	ATATATGGGTCTGGAAGTGTATC	
			C:	ACTGCAGCCTTGAACTCCTG	

*Primer nomenclature is from listed references, with sequence reported 5′→3′. Primers for detection of Hb Quong Sze and termination codon mutations are not included.

$-\alpha^{3.7}$ alleles (Table II) [9]. The exact subtype of the $-\alpha^{3.7}$ allele (I, II, or III) can then be defined by digestion with *Apa*I [7,9,32], giving rise to characteristic bands of 1,600 + 300 bp, 1,900 bp, or 1,800 + 100 bp for forms I, II, or III, respectively.

$-\alpha^{4.2}$

Misalignment and homologous recombination between the 4.2-kb distant homologous X regions gives rise to the $-\alpha^{4.2}$ allele (leftward α-thalassemia-2), which is most prevalent in Southeast Asians [31,33]. This deletion can be detected by a PCR-based method in which selective amplification of normal and $-\alpha^{4.2}$ alleles is achieved using D (or G)/F and D (or G)/E oligonucleotide primer pairs, respectively (Table II) [9].

ASIANS

Most of these alleles have been described either in Asian Indian or Southeast Asian populations, which include Chinese, Thai, Filipino, Cambodian, Vietnamese, and Laotian populations. As the prevalence of α-thalassemia-1 (α^0) is high, Hb H and Hb Bart's hydrops fetalis cause significant public health problems in Southeast Asia. Development of simple PCR-based strategies which screen for the prevalent alleles in specific ethnic groups

TABLE III. Detection of Normal (N) and Mutant (M) α-Globin Gene Alleles by Allele-Specific Oligonucleotide (ASO) Hybridization*

Allele	ASO probe sequence 5′–3′	Reference no.
$\alpha\alpha^{IVS\text{-}I\ nt\ 117\ (G\rightarrow A)}$	N-CAGGAACATCCTGCGGGGA M-TCCCCGCAAGATGTTCCTG	18
$\alpha^{TSaudi}\alpha$	N-TTTGAATAAAGTCTGAGTGGG M-CCCACTCAGACCTTATTCAAA	65
$\alpha^{T}\alpha$	N-TTTGAATAAAGTCTGAGTG M-TTTGAATGAAGTCTGAGTG	15
$\alpha^{Constant\ Spring}\alpha$	N-CAAATACCGTTAAGCTGGAGC M-CAAATACCGTCAAGCTGGAGC	11
$\alpha^{Icaria}\alpha$	N-CTCCAAATACCGTTAAGC M-AAATACCGTAAAGCTGGAG	12
$\alpha^{Codon\ 30/31}\alpha$	N-GCCCTGGAGAGGTGAGGCT M-AGGCCCTGGAGGTGAGGCT	16
$\alpha^{Agri}\alpha$	M-GAGGCCCCGGAGAGGTGA	19
$\alpha^{Adana}\alpha$	N-AGGGCCACGGCAAGAAGGT M-AGGGCCACGACAAGAAGGT	20
$\alpha^{Koya\ Dora}\alpha$	N-AAATACCGTTAAGCTGGAG M-AAATACCGTTCAGCTGGAG	45
$\alpha^{Suan\ Dok}\alpha$	M-CTGGTGACCCGGGCGCC	48

*Underlined bases represent mutation site.

TABLE IV. Nondeletional α-Thalassemia Alleles*

Allele	Altered gene region	Mutation	Geographical distribution	Detection	Reference no.
Asians					
$\alpha^{PA\text{-}2}\alpha$	α_2: Poly A signal	AATAAAG→AATA--G	Indian	ASO	22, 23
	α_1: IVS-I nt 117	CAGGAT→CAAGAT	Indian	ASO	18
$\alpha^{CS}\alpha$	α_2: termination cd	TAA→CAA (Ter→Gln)	SEA	ASO, *Mse*I, *Tru*9I	11, 17, 43
$\alpha^{TAA\rightarrow TAT}\alpha$	α_2: termination cd	TAA→TAT (Ter→Tyr)	SEA	*Mse*I, *Tru*9I	24
$\alpha^{Koya\ Dora}\alpha$	α_2: termination cd	TAA→TCA (Ter→Ser)	Indian	ASO, *Mse*I, *Tru*9I	17, 44, 45
$\alpha^{Suan\ Dok}$	α_2: codon 129	CTG→CGG (Leu→Arg)	SEA	ASO, *Sma*I	47, 48
$\alpha^{TQuong\ Sze}\alpha$	α_2: codon 125	CTG→CCG (Leu→Pro)	SEA	*Msp*I	34
Mediterraneans					
α^{Hph}	α_2: IVS-I donor site	GGTGAGG→GG-----	Mediterranean	*Hph*I	21, 63
$\alpha^{TSaudi}\alpha$	α_2: Poly A signal	AATAAA→AATAAG	Mediterranean, Middle Eastern	ASO	42, 65
$\alpha^{T}\alpha$	α_2: Poly A signal	AATAAA→AATGAA	Turkish	ASO	15
$\alpha^{NcoI}\alpha$	α_2: initiation cd	CCATGG→CCACGG	Mediterranean	*Nco*I	21, 66
$\alpha\alpha^{NcoI}$	α_1: initiation cd	CCATGG→CCGTGG	Mediterranean	*Nco*I	67
$-\alpha^{-3.7\ (NcoI)}$	$-\alpha^{-3.7II}$	ACCATGG→--CATGG	North African, Mediterranean	*Nco*I	68
$\alpha^{Icaria}\alpha$	α_2: termination cd	TAA→AAA (Ter→Lys)	Mediterranean	ASO, *Mse*I, *Tru*9I	12, 17, 69
$\alpha^{Agr}\alpha$	α_2: codon 29	CTG→CCG (Leu→Pro)	Greek	ASO	19
$\alpha^{Adana}\alpha$	α_1: codon 59	CCG→CAG (Gly→Asp)	Turkish	ASO	20
$\alpha^{Petah\ Tikvah}$	α: codon 110	XXX→XXX (Ala→Asp)	Iraqi Jewish		70
$\alpha^{Questemberg}\alpha$	α_2: codon 131	TCT→CCT (Ser→Pro)	Mediterranean		71
Africans					
$-\alpha^{NcoI}$	−α: initiation codon	CCATGG→CCGTGG	African Canadian	*Nco*I	79
$\alpha^{Seal\ Rock}$	α_2: termination cd	TAA→GAA (Ter→Glu)	African American	*Mse*I, *Tru*9I	80, 17
	α_2: codon 116	GAG→TAG (Leu→Ter)	African American		81
	$-\alpha^{3.7}$: codon 30/31	GAGAGG→GAG--G	African American	ASO	82, 16
$\alpha^{Evanston}$	$-\alpha^{3.7}$: codon 14	(Trp→Arg)	African American		83

*ASO, allele-specific oligonucleotide.

is of enormous value in establishing preventive programs in these populations [34].

$-\alpha^{2.7}$ is a 2.7-kb deletion previously identified in a Chinese family which encompasses the entire α_1 gene [35], while $-\alpha^{3.5}$ is a 3.5-kb α-thalassemia-2 deletion described in two unrelated individuals from India. The 5′-deletion breakpoint maps in the Z subsegment of the α_1 gene, while the 3′ breakpoint maps 5′ of the θ_1 gene [36].

$--^{SEA}$ is the most common α-thalassemia-1 variant in Southeast Asia [33,37]. The deletion spans an approxi-

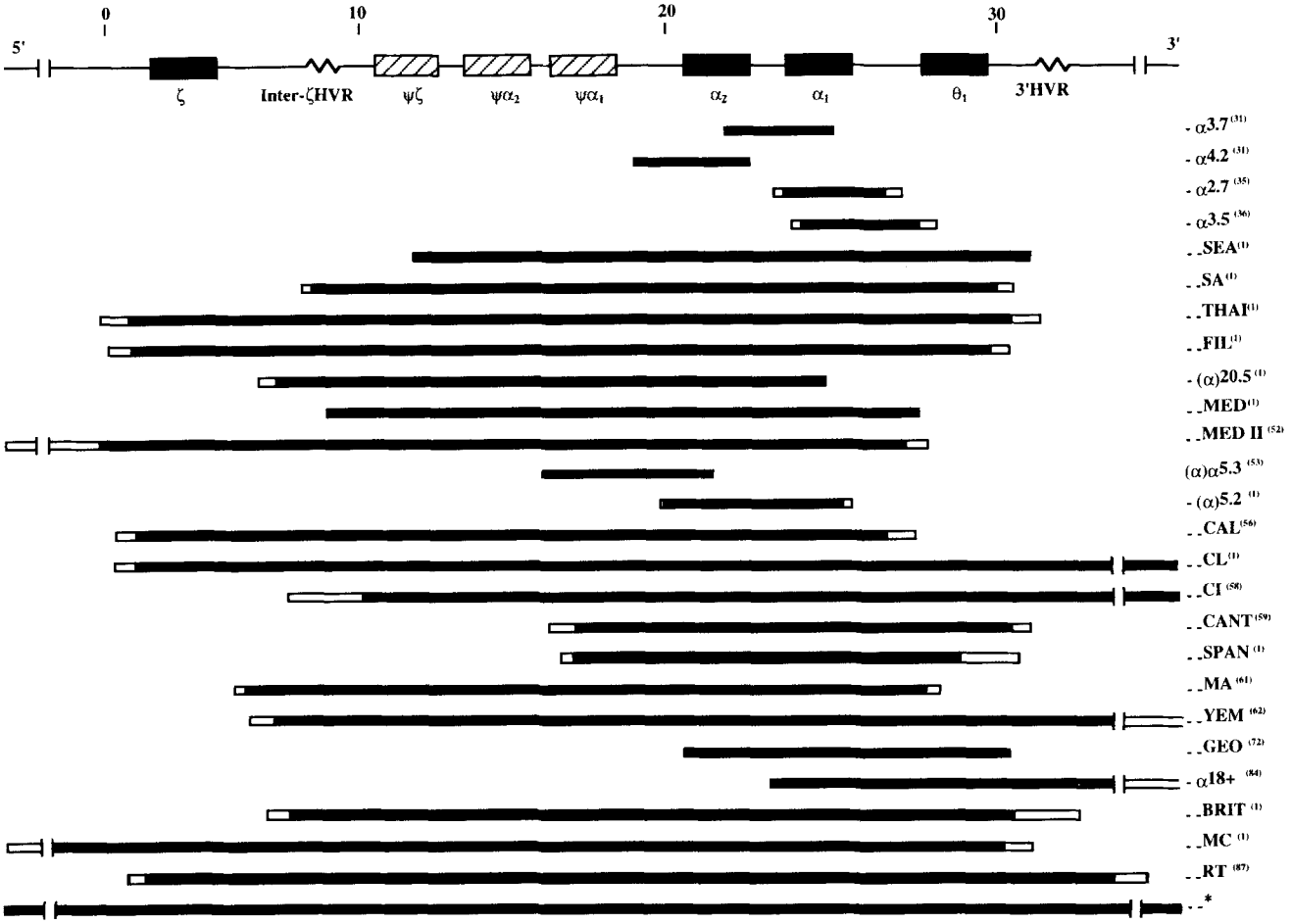

Fig. 1. α^0-thalassemia deletions. Expressed genes in the human α-like globin gene cluster are represented as black boxes, pseudogenes as cross-hatched boxes, and hypervariable regions as zigzag lines at top, with numbers indicating size in kb. Various deletional forms are listed with their names at right, and extent of deletion indicated by solid horizontal bars under cluster. Open areas at ends of some deletions indicate degree of uncertainty for extent of deletion. Deletions removing the α-cluster LCR region are not included. *Group of deletions which removes entire cluster and whose breakpoints are not well-defined [according to refs. 41,60,73–75,88].

mately 20-kb DNA region and removes the $\psi\alpha_2$-, $\psi\alpha_1$-, α_2-, α_1-, and θ_1-globin genes. Selective amplification of normal or mutant alleles is achieved using 7/9 or A/C and 7/8 or A/B pairs of oligonucleotide primers, respectively (Table II) [5,6]. $-^{SA}$ was initially described in a patient from the so-called "Cape Colored" population of South Africa, which includes individuals from diverse ethnic groups such as Africans, Asian Indians, and Europeans. It is likely that it originated from India, as it was further described in a patient from Varya in Northeastern India [38,39]. The deletion removes approximately 23 kb, encompassing a region from 5′ of $\psi\zeta_1$ to 3′ of α_1. $-^{THAI}$ is a 34–38-kb deletion, and was first described in an individual of Thai origin [40]. The deletion extends from 5′ upstream of ζ to 3′ of θ_1. $-^{FIL}$ is a 30–34-kb deletion, described in an individual from the Philippines, and removes all α-like globin genes [40]. $-^{HW}$ is an at least 100-kb deletion described in a Chinese individual, which also removes the entire α-line globin gene cluster [41].

$\alpha^{PA\text{-}2}\alpha$ is a 2-bp deletion at the polyadenylation signal sequence AATAAA [22,23], similar to the $\alpha^{TSaudi}\alpha$ mutation [42], which was described in two different Asian Indian individuals. Detection of $\alpha^{PA\text{-}2}\alpha$ was initially accomplished by SSCA followed by direct sequencing of the amplified DNA fragment [22]. A point mutation at the acceptor splice site (IVS I nt 117) of the α_1 gene has been found in the Asian Indian population at a frequency of 6%. Detection of these two mutations has been achieved with hybridization of the amplified α genes using ASO probes [18, 23] (Table III).

Mutations in the termination condon may result in translation readthrough to the next in-phase termination codon. These mutations can lead to production of a 172-amino-acid-long, unstable, α-globin chain variant. Five

different amino-acid insertions at position 142 have been described, three of which are encountered in Asian populations. Hb Constant Spring is by far the most common α-chain variant in Southeast Asia [43], while Hb Koya Dora is prevalent in Asian Indian patients [44]. The third termination codon mutation encodes a tyrosine residue at position 142 and was described in a Laotian patient [24]. Mutations at the termination codon abolish *Mse*I or *Tru*9I cleavage sites; and thus, detection can be achieved with digestion of an amplified α_2 gene [17]. ASO probes for hybridization of the amplified α_2 gene have also been described for Hb Constant Spring and Hb Koya Dora (Table III) [11,14,45]. Amplification-refractory mutation system (ARMS) and selective amplification of the mutant α_2 gene by asymmetric priming followed by discrimination of normal and mutant alleles by an allele-specific fluorescent PCR reaction have also been used for detection Hb Constant Spring (Table II) [13, 46].

Hb Quong Sze and Hb Suan Dok are α-chain structural variants encountered in Southeast Asia, caused by point mutations leading to highly unstable mutant chains which undergo rapid proteolytic destruction. Hb Quong Sze is caused by a mutation at codon 125, which gives rise to a new *Msp*I cleavage site. Amplification of a segment of the α_2 gene followed by digestion with *Msp*I yields two bands of 289 and 282 bp for the normal allele and three bands of 289, 159, and 123 bp for the mutant allele [34]. A similar strategy can be used for detection of Hb Suan Dok, since this mutation at codon 109 creates a new *Sma*I cleavage site [47]. ASO probes for detection of this mutation have also been described (Table III) [48].

MEDITERRANEANS

This group includes alleles described in populations living in the countries bordering the Mediterranean Sea. Population migration in this area is a common sociological phenomenon and has led to dispersion of α-thalassemia variants which are frequent in the Mediterraneans.

$-(\alpha)^{20.5}$ and $-^{MED}$ are the most common Mediterranean deletions. $-(\alpha)^{20.5}$ removes a 20.5-kb region which extends from 5′ of $\psi\zeta_1$ to codon 51 of the α_1-globin gene [49,50]. The 5′ breakpoint for $-^{MED}$ is located 5′ of $\psi\zeta_1$, and the 3′ breakpoint maps just 5′ of θ_1 [51]. Selective amplification of the normal allele is accomplished using the oligonucleotide primer pairs 2/3 or 5/6, while the $-(\alpha)^{20.5}$ and $-^{MED}$ alleles are detected following selective amplification using 1/3 and 4/6 primer pairs, respectively (Table II) [6]. The 3′ breakpoint of $-^{MED\text{-}II}$, which is a >26.5-kb deletion, maps close to that of $-^{MED}$, whereas the 5′ breakpoint maps upstream of ζ [52].

$(\alpha)\alpha^{5.3}$ is an α-thalassemia-2 deletion which was described in a family from Southern Italy and probably arose from an intrachromatid illegitimate recombination event involving *Alu* repeats [53]. $-(\alpha)^{5.2}$ is the smallest α-thalassemia-1 deletion, removing α_2 and a portion of the α_1-globin gene. It was originally described in a Greek patient [54] and, subsequently, was further characterized in an Italian family using a PCR-based assay with primers flanking the breakpoint (Table II) [55].

$-^{CAL}$ is an ~32-kb deletion first detected in a family from Calabria, Italy [56], but also recently described in a Spanish family [57]. The 5′ breakpoint of the deletion is located upstream of ζ, while the 3′ breakpoint maps between α_1–θ_1. Five other deletions have so far been detected in isolated Spanish families. $-^{CI}$ is a 27-kb deletion, whose 5′ breakpoint is located between ζ–ψζ, while the 3′ breakpoint maps downstream of the 3′-HVR region [58]. $-^{CANT}$ and $-^{SPAN}$ are two deletions, ~14- and ~11-kb, respectively, which remove both α genes [59,60]. $-^{MA}$ is a 22-kb deletion which extends from 3′ of ζ to 3′ of α_1, and was detected in a chromosome carrying a ζ-gene triplication [61]. The fourth deletion, $-^{BR}$, is an at least 100-kb deletion encompassing the entire α-like globin gene cluster. [60].

$-^{YEM}$ is a large deletion of at least 39 kb, detected in 4 unrelated patients from the Yemenite community of Israel. The 5′ breakpoint is located between ζ and the Iζ-HVR region, while the 3′ breakpoint maps downstream of the 3′-HVR, possibly at coordinate 43.3 [62].

Two large deletions that encompass the upstream α-cluster LCR region have been described. $(\alpha\alpha)^{IdF}$ is an α^0-thalassemia variant first described in an Italian patient due to truncation of the short arm of chromosome 16. Gene-blotting studies with *Xba*I or *Bgl*II and hybridization with a Iζ-HVR-specific probe produces smeared bands, which are indicative of a deletion extending to the telomere [29]. $(\alpha\alpha)^{MM}$ is an at least 105.5-kb deletion, detected in an individual from the Azores Islands, Portugal, which maps 5′ of ζ and extends to the telomere of 16p [28].

$\alpha^{Hph}\alpha$ is a pentanucleotide deletion in the splice donor site of IVS-I and was first described in an Italian patient [63,64]. This mutation abolishes an *Hph*I site in the α_2 gene, and digestion of amplified α_2 gene with *Hph*I yields 657-, and 242- and 170-bp bands for this allele, compared with 334-, 323-, 242-, and 170-bp bands for the normal allele (Tables II and IV) [21].

$\alpha^{TSaudi}\alpha$ (or $\alpha^{PA}\alpha$) and $\alpha^{T}\alpha$ are two nondeletional forms of α-thalassemia caused by base substitutions in the highly conserved polyadenylation signal sequence AATAAA, located 10–30 nucleotides upstream from the mRNA polyadenylation site, which facilitates endonucleolytic cleavage and poly A addition to the 3′ end of mRNAs [15,42,65]. These mutations abolish production of normal mRNA from α_2 and possibly also decrease expression of the adjacent α_1-globin gene. The $\alpha^{TSaudi}\alpha$ mutation, which was also found in both α_2-globin genes on a chromosome with a triplicated α-globin gene locus, was initially described in Saudi Arabia and subsequently

in different areas of the Mediterranean basin, while $\alpha^T\alpha$ was described in a familiy from Turkey [15]. Detection of $\alpha^{TSaudi}\alpha$ and $\alpha^T\alpha$ is accomplished by hybridization of the PCR-amplified α_2 gene with ASO probes (Table III) [15,65]. $\alpha^{TSaudi}\alpha$ can also be detected by selective amplification of the mutant allele with C8 and SPA primers, using a PCR-amplified α_2 gene as a template (Table II) [19].

Two nondeletional forms of α-thalassemia, $\alpha^{NcoI}\alpha$ and $\alpha\alpha^{NcoI}$, encountered in many Mediterranean populations, are caused by base substitutions in the translational initiation codon ATG in the α_2 or α_1 gene, respectively, which presumably completely abolish translation [66,67]. A third mutation, found on a $-\alpha^{3.7II}$ chromosome and described in an Algerian patient, contained a 2-bp deletion at positions −2 and −1, and reduced mRNA translation by 30–50% [68]. Since $\alpha^{NcoI}\alpha$ and $\alpha\alpha^{NcoI}$ abolish an *Nco*I site, digestion of appropriate PCR products with *Nco*I shows no cleavage for the mutant allele and generation of two fragments for the normal allele. Using α_2-specific primer pairs, a 1,073-bp fragment is generated and not digested by *Nco*I for the α^{NcoI} allele, while 245-bp and 828-bp bands are seen following digestion of the normal allele (Tables II and IV) [21].

Hb Icaria was described in Eastern Mediterranean populations and is an unstable enlongated, α-globin chain which contains lysine at position 142, caused by a base substitution in the termination codon [69]. Detection can be achieved by hybridization of the PCR-amplified α_2 gene with ASO probes (Table III) [12]. Nonspecific screening for all termination codon mutations can be done following digestion with *Mse*I or *Tru*9I [17].

A number of unstable α-chain variants have been described in isolated Mediterranean individuals. These mutations result in production of highly unstable chains which are rapidly removed from erythroid cells. Hb Adana and Hb Agrinion ($\alpha^{Agr}\alpha$), described in Turkish and Greek patients, are caused by mutations at codons 59 and 29, respectively [19,20]. Both mutations can be detected by ASO hybridization to the PCR-amplified α_2 gene (Table III). $\alpha^{Agr}\alpha$ can also be detected by selective amplification of the mutant allele (Table II) [20]. Hb Petah Tikvah is caused by a mutation at codon 110, and was described in 2 Iraqi Jewish patients living in Israel [70]. Mutation at codon 131 of the α_2 gene leads to the production of Hb Questembert, which was detected in a patient from the former Yugoslavia who presented with a decreased α/β globin chain synthetic ratio [71].

AFRICANS

Although deletion of one α-globin gene is very common among Africans, α-thalassemia-1 (α^0) is very rare. Thus, hydrops fetalis is rarely encountered in this population.

Four large deletions have been described in African Americans. $-^{GEO}$ is an 8.5-kb deletion which removes both α-globin genes and the θ_1 gene [72]. Two deletions, one at least 80 kb and the other at least 125 kb, remove all α-like globin genes, but their breakpoints are not well-characterized [73–75]. The third deletion was described in a girl with mild mental retardation and Hb H disease, and may represent one of those deletions involving chromosome band 16p13.3, which is often associated with α-thalassemia/mental retardation syndromes [76,77]. The fourth deletion may likely be identical to $-^{BRIT}$ [78].

A nondeletional form of α-thalassemia involving a base substitution in the initiation codon found on one $-\alpha$ chromosome was described in an African Canadian patient [79], and may be related to the $\alpha^{NcoI}\alpha$-thalassemia variant found in Italy [66]. This mutation abolishes an *Nco*I site, and can be inferred following PCR amplification of the mutant α gene using C1/C9c primers and digestion with *Nco*I, which yields one band of 1,091 bp instead of 245- and 846-bp bands for the normal allele (Tables II and IV) [4]. A similar mutation described in an Algerian patient is discussed under the Mediterranean alleles.

Hb Seal Rock is an elongated, unstable, α-like globin chain with a serine residue at position 142, similar to Hb Constant Spring, described in an African family [80]. The mutation is detected, as with Hb Constant Spring, following digestion of the PCR-amplified α_2-gene region with *Mse*I or *Tru*9I [17].

Two alleles involving mutations that affect mRNA translation have been identified in African American families: one due to mutation at codon 116 in α_2, giving rise to a premature termination codon [81]; and the second due to a dinucleotide deletion at codons 30/31 in the α gene on one $-\alpha^{3.7}$ chromosome, which causes a reading-frame shift and generation of a new out-of-frame termination codon at position 55 in the altered peptide [82]. ASO probes for detection of this mutation have been described (Table III) [16].

Hb Evanston is a highly unstable α-chain variant caused by a point mutation at codon 14 in the α gene on one $-\alpha^{3.7}$ chromosome, and was detected in two unrelated African American families [83].

NORTHERN EUROPEANS

Although α-thalassemia syndromes are rare in Northern Europe, several distinctive variants have been identified in isolated Northern European families.

$-\alpha^{18+}$ is an α-thalassemia-2 deletion described in a Czechoslovakian family, and removes >18 kb encompassing the α_1 and θ_1 genes [84]. $-^{BRIT}$ is an ~26-kb deletion, described in 8 British individuals [78,85], while $-^{MC}$ is a large deletion of ~43 kb detected in a British family. The 5′ breakpoint of $-^{MC}$ is located upstream of ζ, while the 3′ breakpoint maps 3′ of θ_1 [86]. $-^{RT}$ is a

36.5–40-kb deletion detected in a patient of British and German descent, which removes all the α-like globin genes [87]. An α-thalassemia-1 deletion was also described in a family of German descent, which removed >47 kb and encompassed the entire α-like globin gene cluster [88].

Three large deletions remove the LCR region and inhibit expression from the entire downstream α-like globin gene cluster. $(\alpha\alpha)^{TI}$ and $(\alpha\alpha)^{RA}$ were detected in British individuals [25,26], while $(\alpha\alpha)^{IJ}$ was detected in a German individual [27].

De novo large deletions involving chromosome band 16p13.3, which remove the entire α-globin gene cluster, have been identified in 4 individuals from the United Kingdom and 4 others from different parts of the world. These patients were diagnosed as having α-thalassemia/mental retardation syndromes with mild-to-moderate mental handicap, frequently dysmorphic features, and α-thalassemia of variable severity [76,77]. X-linked α-thalassemia/mental retardation syndrome (ATR-X syndrome) seems to be caused by mutations to the XH2 gene located within Xq13.1–q21.1, which may downregulate expression of several genes, including the structurally-intact normal α-like globin gene cluster [89].

CONCLUSION

The armory for detection of the molecular defects causing α-thalassemia has been enriched enormously since the advent of PCR technology. A variety of simple, rapid, PCR-based methods are currently available for detection of the most common α-thalassemia mutations. Still, rare defects can only be characterized with restriction analysis, gene mapping, and/or DNA sequence analysis. Defects are vastly heterogeneous but generally ethnic-specific; and, thus, research and clinical laboratories need to develop population-oriented strategies to simplify detection for diagnostic, genetic counseling, or thalassemia research purposes.

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